Aedes aegypti (Diptera: Culicidae) Biting Deterrence: Structure-Activity Relationship of Saturated and Unsaturated Fatty Acids

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ABSTRACT In this study we evaluated the biting deterrent effects of a series of saturated and unsaturated fatty acids against *Aedes aegypti* (L), yellow fever mosquito (Diptera: Culicidae) using the K & D bioassay module system. Saturated ($C_{6:0}$ to $C_{16:0}$ and $C_{18:0}$) and unsaturated fatty acids ($C_{11:1}$ to $C_{14:1}$, $C_{16:1}$, $C_{18:1}$, and $C_{18:2}$) showed biting deterrence index (BDI) values significantly greater than ethanol, the negative control. Among the saturated fatty acids, mid chain length acids ($C_{10:0}$ to $C_{13:0}$) showed higher biting deterrence than short ($C_{6:0}$ to $C_{9:0}$) and long chain length acids ($C_{14:0}$ to $C_{18:0}$), except for $C_{8:0}$ and $C_{16:0}$ that were more active than the other short and long chain acids. The BDI values of mid chain length acids ($C_{10:0}$ to $C_{13:0}$) were not significantly less than *N*, *N*-diethyl-*meta*-toluamide (DEET), the positive control. Among the unsaturated fatty acids, $C_{11:1}$ showed the highest activity (BDI = 1.05) and $C_{18:2}$ had the lowest activity (BDI = 0.7). In $C_{11:1}$, $C_{12:1}$, and $C_{14:1}$ BDI values were not significantly less than DEET. After the preliminary observations, residual activity bioassays were performed on $C_{11:0}$, $C_{12:0}$, $C_{11:1}$, and $C_{12:1}$ over a 24-h period. All the fatty acids ($C_{11:0}$, $C_{12:0}$, $C_{11:1}$, and $C_{12:1}$) and DEET showed significantly higher activity at all test intervals than the solvent control. At treatment and 1-h posttreatment, all fatty acids showed proportion not biting (PNB) values not significantly less than DEET. At 3-, 6-, and 12-h posttreatment, all fatty acids showed PNB values significantly greater than DEET. At 24-h posttreatment, only the PNB value for $C_{12:0}$ was significantly higher than DEET. The dose–responses of $C_{12:0}$ and DEET were determined at concentrations of 5–25 nmol/cm^2. As in the residual activity bioassays, the PNB values for $C_{12:0}$ and DEET at 25 nmol/cm^2 were not significantly different. However, at lower concentrations, the PNB values for $C_{12:0}$ were significantly greater than DEET. These results clearly indicate that mid chain length fatty acids not only have levels of biting deterrence similar to DEET at 25 nmol/cm^2 in our test system, but also appeared to be more persistent than DEET. In contrast, in vivo cloth patch assay system showed that the mid-chain length fatty acids, $C_{11:0}$, $C_{11:1}$ $C_{12:0}$, and $C_{12:1}$ had minimum effective dose (MED) values greater than DEET against *Ae. aegypti* and their relative repellency varied according to species tested. The MED values of 120 ($C_{11:0}$), 145 ($C_{12:0}$) and 116 ($C_{11:1}$) nmol/cm^2 against *Anopheles quadrimaculatus* Say, indicated that these acids were not as potent as DEET with a MED of 54 nmol/cm^2. The MED ratio of the $C_{11:0}$ and $C_{11:1}$ for all three mosquito species indicated the C_{11} saturated and unsaturated acids as more repellent than their corresponding $C_{12:0}$ and $C_{12:1}$ homologues.

KEY WORDS fatty acid, biting deterrence, repellent, structure-activity relationship, *Aedes aegypti*

Mosquitoes transmit pathogens that cause serious human diseases including malaria, Japanese encephalitis, yellow fever, dengue, and filariasis. *Aedes aegypti* (L.) (Diptera: Culicidae) carries arboviruses, which cause dengue fever in human beings; and 40% of the world population is considered to be at risk (Dowlathabad et al. 2009). The use of insecticides is a main method for management of mosquito populations. Insecticides from various chemical groups are commonly used to control these vectors, but mosquitoes are evolving resistance to some of these chemicals, exposing humans to populations of more difficult to manage insects (Hoel et al. 2010). Hence, identifying novel compounds for mosquito control is a priority. Products derived from plants might yield alternatives in this regard, whether as insecticides, growth and reproductive inhibitors, repellents, and oviposition deterrents (Sukumar et al. 1991).

Plant products (e.g., fruit and seeds) can offer a rich source of fatty acids some of which seem to act as biting deterrents against mosquitoes (Cantrell et al. 2011, Jones et al. 2012). However, information on the behavioral effects of such compounds is relatively

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14. ABSTRACT

In this study we evaluated the biting deterrent effects of a series of saturated and unsaturated fatty acids against Aedes aegypti (L), yellow fever mosquito (Diptera: Culicidae) using theK&Dbioassay module system. Saturated(C6:0 toC16:0 andC18:0) and unsaturated fatty acids(C11:1 to C14:1, C16:1, C18:1, and C18:2) showed biting deterrence index (BDI) values signi?cantly greater than ethanol, the negative control. Among the saturated fatty acids, mid chain length acids (C10:0 to C13:0) showed higher biting deterrence than short (C6:0 to C9:0) and long chain length acids (C14:0 to C18:0) except for C8:0 and C16:0 that were more active than the other short and long chain acids. The BDI values of mid chain length acids (C10:0 to C13:0) were not signi?cantly less than N, N-diethyl-metatoluamide (DEET), the positive control. Among the unsaturated fatty acids, C11:1 showed the highest activity (BDI 1.05) andC18:2 had the lowest activity (BDI 0.7). InC11:1, C12:1, andC14:1 BDI values were not signi?cantly less than DEET. After the preliminary observations, residual activity bioassays were performed onC11:0, C12:0, C11:1, andC12:1 over a 24-h period. All the fatty acids (C11:0, C12:0, C11:1 and C12:1) and DEET showed signi?cantly higher activity at all test intervals than the solvent control. At treatment and 1-h posttreatment, all fatty acids showed proportion not biting (PNB) values not signi?cantly less than DEET. At 3-, 6-, and 12-h posttreatment, all fatty acids showed PNB values signi?cantly greater than DEET. At 24-h posttreatment, only the PNB value for C12:0 was signi?cantly higher than DEET. The dose?responses ofC12:0 and DEET were determined at concentrations of 5?25 nmol/cm2. As in the residual activity bioassays, the PNB values for C12:0 and DEET at 25 nmol/cm2 were not signi?cantly different. However, at lower concentrations, the PNB values for C12:0 were signi?cantly greater than DEET. These results clearly indicate that mid chain length fatty acids not only have levels of biting deterrence similar to DEET at 25 nmol/cm2 in our test system, but also appeared to be more persistent than DEET. In contrast, in vivo cloth patch assay system showed that the mid-chain length fatty acids,C11:0,C11:1C12:0, andC12:1 had minimum effective dose(MED)values greater than DEET against Ae. aegypti and their relative repellency varied according to species tested. The MED values of 120 (C11:0), 145 (C12:0) and 116 (C11:1) nmol/cm2 against Anopheles quadrimaculatus Say, indicated that these acids were not as potent as DEET with a MED of 54 nmol/cm2. The MED ratio of the C11:0 and C11:1 for all three mosquito species indicated the C11 saturated and unsaturated acids as more repellent than their corresponding C12:0 and C12:1 homologues.

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scant. Fatty acids have been studied by some researchers in the past for their repellent properties. Skinner et al. (1970) studied the human skin surface lipid fatty acids and reported that these lipids contain components repellent to female *Ae. aegypti.* Subsequent work by Bosch et al. (2000) showed that some saturated fatty acids, including undecanoic acid were repellent to *Ae. aegypti;* and Reifenrath (2005) found that mixtures of unsaturated short chain acids combined with some saturated acids were repellent to stable flies. None of these authors systematically evaluated the effect of chain length on the potency of these acids. In the current study, we evaluated both saturated and unsaturated pure fatty acids of different chain length for their biting deterrent activity against *Ae. aegypti.* Highly active fatty acids from a mid-chain length group were further tested to evaluate their persistence after application. The most active compounds were further evaluated in studies using human subjects and three species of mosquitoes: *Ae. aegypti* L, *Anopheles albimanus* Wiedemann and *An. quadrimaculatus* Say.

Materials and Methods

Chemicals. The saturated fatty acids hexanoic acid ($C_{6:0}$), heptanoic acid ($C_{7:0}$), octanoic acid ($C_{8:0}$), nonanoic acid ($C_{9:0}$), tridecanoic acid ($C_{13:0}$), and unsaturated fatty acids undecanoic acid ($C_{11:1}$), 11-dodecenoic acid ($C_{12:1}$), 12-tridecenoic acid ($C_{13:1}$), myristoleic acid ($C_{14:1}$), and palmitoleic acid ($C_{16:1}$) were all purchased from Nu-Chek Prep, Inc. (Elysian, MN). Decanoic acid ($C_{10:0}$), undecanoic acid ($C_{11:0}$), dodecanoic acid ($C_{12:0}$), tetradecanoic acid ($C_{14:0}$), hexadecanoic acid ($C_{16:0}$), octadecanoic acid ($C_{18:0}$), oleic acid ($C_{18:1}$), and linoleic acid ($C_{18:2}$) were all purchased from Sigma–Aldrich (St. Louis, MO).

Insects. *Ae. aegypti* used in Klun & Debboun (K & D) bioassays were from a laboratory colony maintained using standard procedures (Pridgeon et al. 2007) since 1952 at the Mosquito and Fly Research Unit at the Center for Medical, Agricultural and Veterinary Entomology, United States Department of Agriculture, Agriculture Research Service, Gainesville, FL. We received the eggs and stored these in our laboratory (Biological Field Station, The University of Mississippi, Abbeville, MS) until needed. Mosquitoes were reared to the adult stage by feeding the larvae on a diet of 2:1 alfalfa pellets (U.S. Nutrition Inc., Bohemia, NY) and hog chow (Ware Milling, Houston, MS). The diet contents were ground and passed through sieve no. 40, 425 micron (USA Standard Sieve, Humboldt MFG Co., Norridge, IL). The eggs were hatched by placing a piece of a paper towel with eggs in a cup filled with 100 ml de-ionized water containing a small quantity of larval diet and maintained under vacuum (≈1 h). Larvae were removed from vacuum and held overnight in the cup. These larvae were then transferred into 500-ml cups (≈50–100 larvae per cup) filled with water. Larval diet was added every day until pupation, and the mosquitoes were kept in an environment controlled room. Both the larvae and adults were maintained at a temperature of 27 ± 2°C and 70 ± 5% RH in a photoperiod regimen of 12:12 (L: D) h. The adults were fed on cotton pads moistened with 10% sucrose solution placed on the top of screens of 4 liter cages. Seven to 14-d-old mated females used in these bioassays were deprived of sucrose for 24 h before the test; but had free access to water-soaked cotton.

Mosquito Biting Bioassays. Bioassays were conducted using a six-celled in vitro K & D module bioassay system developed by Klun et al. (2005) for quantitative evaluation of biting deterrent properties of candidate compounds. Here we use feeding deterrent in the sense of Dethier (1960), that is, a chemical that inhibits feeding when present in a place where the insects feed in its absence. This is contrasted by repellent, which is a chemical that causes insects to make oriented movement away from its source. The K & D system consists of a six-well reservoir with each of the 4 × 3 cm wells containing 6 ml of feeding solution. As reported earlier (Klun et al. 2008), female mosquitoes fed as well on the CPDA-1 (citrate-phosphate-dextrose-adenine) + ATP feeding solution as they did on blood. Therefore, we used the CPDA-1 + ATP solution instead of human blood. CPDA-1 was prepared by dissolving 3.33 g sodium citrate, 0.376 g citric acid, 4.02 g dextrose, 0.28 g monobasic sodium phosphate (Fisher Chemical Co., Fairlawn, NJ), and 0.346 g of adenine (Sigma–Aldrich) in 1,026 ml of de-ionized water (American Association of Blood Banks 2005). ATP was added to CPDA-1 to yield 1×10^{-3} M ATP. CPDA-1 and ATP preparations were freshly made on the day of the test and contained a red dye that allowed for identification of mosquitoes that had fed on the solution (see below). DEET (97% purity *N,N*-diethyl-*meta*-toluamide) was obtained from Sigma–Aldrich and used as a positive control. Molecular biology grade ethanol was obtained from Fisher Chemical Co. (Fairlawn, NJ). All fatty acids and DEET treatments were prepared in ethanol. All fatty acids and the positive control DEET were tested at a concentration of 25 nmol/cm^2 except in dose–response study. The stock solutions were kept in a refrigerator at 3–4°C. Treatments were prepared fresh at the time of bioassay.

During the bioassay, temperature of the solution in the reservoirs covered with a collagen membrane was maintained at 37.5°C by circulating water through the reservoir with a temperature-controlled circulatory bath. This CPDA-1 + ATP solution membrane unit simulated a human host for mosquito feeding. The test compounds and controls were randomly applied to six 4 × 3 cm marked portions of nylon organdy strip, which was positioned over the six, membrane-covered wells. A Teflon separator was placed between the treated cloth and the six-celled module. A six-celled K & D module containing five females per cell was positioned over the six wells, trap doors were opened and mosquitoes allowed access at the module for a 3 min period, after which they were collected back into the module. Mosquitoes were squashed and the presence of red dye (or not) in the gut was used as an indicator of feeding. A replicate consisted of six treatments: four

test compounds, DEET (a standard bite deterrent compound) and 95% ethanol as solvent control. DEET at 25 nmol/cm^2 cloth dose was used as a standard because it suppresses mosquito biting by 80% as compared with controls (Klun et al. 2005). Five replicates were conducted per day using new batches of mosquitoes for each. Total 15 replications were conducted for each treatment. Bioassays were conducted between 13:00 and 16:00 h.

In persistence studies, organdy strips were treated as described and hung in the laboratory (26 ± 2°C with 40–50% RH). These were then tested at 0-, 1-, 3-, 6-, 12-, and 24-h posttreatment. In the dose–response study, organdy strips were treated with 5–25 nmol/cm^2 concentrations of $C_{12:1}$ or DEET. A solvent control was used in each set of treatments and data were collected as described above.

Repellency Bioassays. Repellency was determined as the minimum effective dosage (MED) of a compound necessary to prevent bites through the fabric. A 0.15 g quantity of each acid ($C_{11:0}$, $C_{12:0}$, $C_{11:1}$, and $C_{12:1}$) or DEET standard were added to 2 ml of acetone in a 7.1 ml screw top glass vial. This stock solution was serially diluted to produce the following test concentrations: 1.5, 0.75, 0.375, 0.187, 0.094, 0.047, 0.023, 0.011, and 0.006 mg/cm^2. For DEET, the dose range in nanomolar amounts was 7,841 down to 31 nmol/cm^2. Muslin cloth was cut into a 50 cm^2 (5 × 10 cm) pieces, rolled lengthwise and placed into each vial. Vials were then sealed and stored in a laboratory freezer until just before testing.

Before experiments, pieces of treated cloth were removed from the vials and stapled onto card stock tabs (5 × 3 cm). These were hung on a drying rack for 3–5 min. Before the start of the assay, study participants used latex gloves to pull a nylon stocking over their arm. A Velcro-sealed vinyl sleeve was then placed over the forearm. The sleeve had a 32-cm^2 (4 × 8 cm) window to allow attractive skin odors to escape and draw mosquitoes to that open area. The purpose of the nylon stocking was to produce a barrier between the dried cloth and the skin, thereby avoiding direct contact of chemical to skin. The dried cloth assembly was affixed over the opening in the sleeve and held in place with masking tape. Participants then inserted their arm with the sleeve and patch into a screened cage that contained 500 ± 50 female *Ae. aegypti*, *An. albimanus*, or *An. quadrimaculatus*. The mosquitoes in each cage were loaded 15–30 min prior from a draw box designed to select host-seeking females (Posey and Schreck 1981). Tests were conducted on each control or treated patch for 1 min. A control patch (acetone solvent only) was tested before the start of experiments and after every 10 tests. If fewer than five landings occurred on the control patch in 30 s, then tests were discontinued for 60 min. At the conclusion of testing, the control patch was tested again. If five landings were not received within 30 s, the data for the replicate was discarded. When testing a patch treated with a candidate repellent, if ≈1% or five mosquito bites were received during this one min test, this compound was considered to have failed, that is, was not repellent at that concentration. If a treated cloth patch received 0–4 bites within a minute, then it was considered as passed, that is, repellent at that concentration of the test compound. The 0.094 mg/cm^2 concentration was tested first for each compound, and then tests were continued with successively higher or lower concentrations depending upon whether this patch failed or passed, respectively.

The time interval between each tested patch was <90 s until 10 successive tests had been conducted. If appreciable mortality occurred over this period additional female mosquitoes were added to the cage to keep the available mosquitoes at ≈500. The estimate of the MED was the lowest concentration that passed for each candidate. Observed MED values for each candidate compound were averaged across participants and reported as a mean MED ± SE. Additional explanation of this type of bioassay can be found in Katritzky et al. (2010).

All volunteers provided informed consent to participate. In total, three male volunteers, ages 35, 36, and 44, participated in this study; each tested each compound once until a consecutive pass/fail result had been achieved and this consisted of one replicate. For *Ae. aegypti*, each volunteer completed three replicates ($N = 9$). For *An. quadrimaculatus*, data from two replicates had to be discarded ($N = 7$), and for *An. albimanus*, the data for one replicate from each person ($N = 6$; all from the final set) had to be discarded. The protocol was approved by the University of Florida Human Use Institutional Review Board (IRB 636-2005).

Data Analyses. Proportion not biting (PNB) was calculated using the following formula:

$$\text{PNB} = 1 - \left(\frac{\text{Total number of females biting}}{\text{Total number of females}}\right)$$

Because the K & D module bioassay system can handle only four treatments along with negative and positive controls, to make direct comparisons among more than four test compounds and to compensate for variation in overall response among replicates, repellency was quantified as Biting Deterrence Index (BDI). The BDIs were calculated using the following formula:

$$[BDI_{i,j,k}] = \left[\frac{PNB_{i,j,k} - PNB_{c,j,k}}{PNB_{d,j,k} - PNB_{c,j,k}}\right]$$

Where $PNB_{i,j,k}$ denotes the proportion of females not biting test compound i for replication j and day k (i = 1–4, j = 1–5, k = 1–2), $PNB_{c,j,k}$ denotes the proportion of females not biting the solvent control for replication j and day k (j = 1–5, k = 1–2) and $PNB_{d,j,k}$ denotes the proportion of females not biting in response to DEET (positive control) for replication j and day k (j = 1–5, k = 1–2). This formula makes an adjustment for interday variation in response and incorporates information from the solvent control as well as the positive control.

A BDI value of 0 indicates an effect similar to ethanol while a value significantly >0 indicates biting

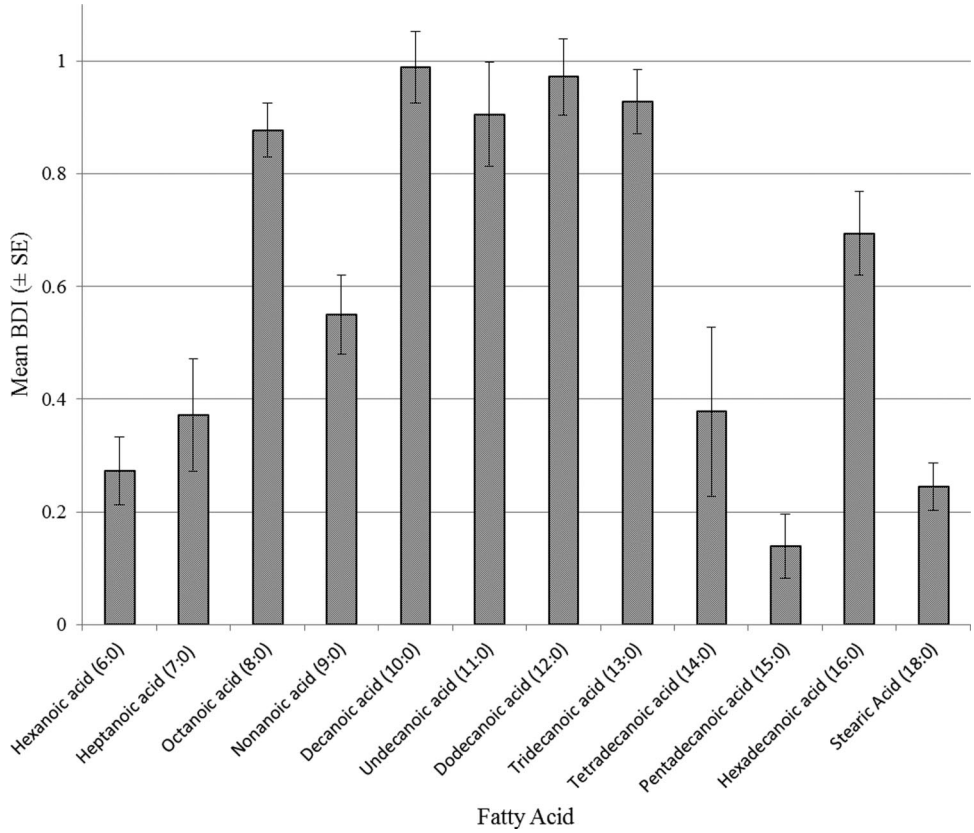

Fig. 1. Mean BDI (±SE) values for *Aedes aegypti* in response to saturated fatty acids. All the fatty acids were tested at 25 nmol/cm^2 and evaluated using DEET at 25 nmol/cm^2 as positive control and ethanol as solvent control. A BDI value >0 indicates deterrence relative to ethanol, and a BDI value not significantly different from one shows deterrence statistically similar to DEET.

deterrent effect relative to ethanol. BDI values not significantly different from one are statistically similar to DEET. BDI values were analyzed using SAS Proc analysis of variance (ANOVA), (SAS Institute 2007), and means were separated using the Ryan–Einot–Gabriel–Welsch Multiple Range Test.

Results

BDI values for the relative biting deterrent effects of saturated fatty acids ($C_{6:0}$ to $C_{18:0}$) against female *Ae. aegypti* are shown in Fig. 1. All saturated fatty acids showed biting deterrence and feeding was significantly less than ethanol control. Short chain length fatty acids ($C_{6:0}$ to $C_{9:0}$) showed significantly lower activity than the mid chain length acids ($C_{10:0}$ to $C_{13:0}$) except $C_{8:0}$ that showed activity similar to $C_{10:0}$ to $C_{13:0}$. Long chain length acids ($C_{14:0}$ to $C_{18:0}$) also showed significantly less biting deterrent activity than the mid chain length acids except $C_{16:0}$ that showed activity similar to $C_{10:0}$ to $C_{13:0}$. $C_{10:0}$, $C_{11:0}$, $C_{12:0}$, and $C_{13:0}$ with mean BDI values of 0.99, 0.91, 0.97, and 0.93, respectively, showed activity that was statistically similar to DEET at 25 nmol/cm^2.

BDI values for unsaturated fatty acids ($C_{11:1}$ to $C_{18:2}$) against female *Ae. aegypti* were significantly higher than for ethanol (Fig. 2). $C_{11:1}$ (BDI value = 1.05) was the most active compound and activity of $C_{12:1}$ and $C_{14:1}$, with BDI values of 0.98, and 0.99, respectively, were statistically similar to $C_{11:1}$. $C_{18:2}$ with a BDI value of 0.70 had the lowest activity but was statistically similar to $C_{13:1}$, $C_{16:1}$, and $C_{18:1}$ with all four compounds showing activities significantly ($P < 0.05$) less than DEET.

Having established that $C_{11:0}$, $C_{12:0}$, $C_{11:1}$, and $C_{12:1}$ had BDI values similar to DEET, we further tested these compounds to quantify their residual activity by exposing mosquito females to treated organdy cloth at various posttreatment time intervals. All treatments showed significantly higher activity at all test intervals than solvent control (Fig. 3). At 0- and 1-h posttreatment, treatments showed activity similar to DEET. At 3-, 6-, and 12-h postapplication, all fatty acids tested showed significantly higher activity than DEET ($P \leq 0.05$). At 24-h posttreatment, only the activity of $C_{12:0}$ was significantly higher ($P \leq 0.05$) than DEET. The increased activity of fatty acids compared against DEET over parts of the test period deserves attention.

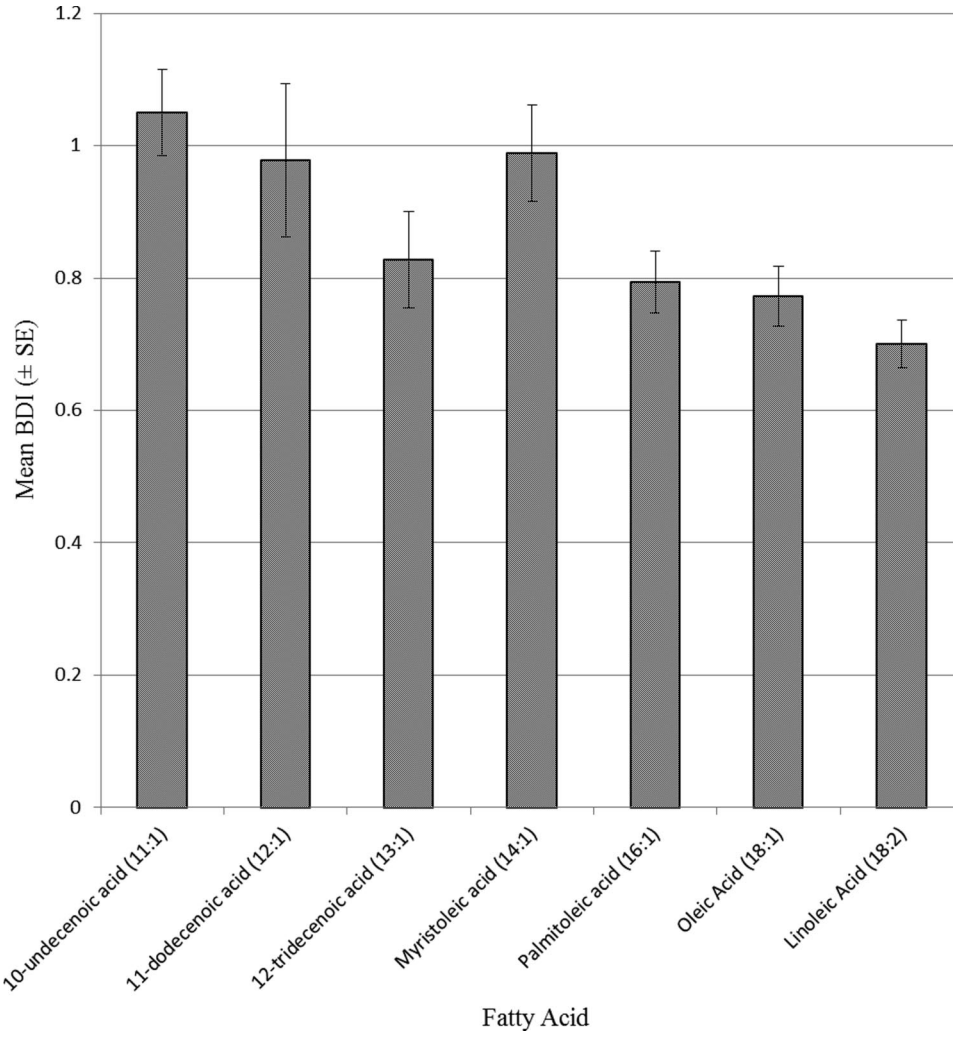

Fig. 2. Mean BDI (±SE) values for *Aedes aegypti* in response to unsaturated fatty acids. All the fatty acids were tested at 25 nmol/cm^2 and evaluated using DEET at 25 nmol/cm^2 as positive control and ethanol as solvent control. A BDI value >0 indicates deterrence relative to ethanol, and a BDI value not significantly different from one shows deterrence statistically similar to DEET.

The dose–response data of $C_{12:0}$ and DEET (Fig. 4) suggest that the higher activity of $C_{12:0}$ compared with DEET (Fig. 3) was due, at least in part, to a higher biting deterrency of $C_{12:0}$ than DEET at a range of test concentrations below the standard concentration of 25 nmol/cm^2. Data at 25 nmol/cm^2 in dose–response study corroborated the data in all the bioassays where we used DEET as a positive control and $C_{12:0}$ as a treatment. At 20, 15, 10, and 5 nmol/cm^2, $C_{12:0}$ showed significantly higher biting deterrent activity than DEET. At the concentration of 10 and 5 nmol/cm^2 DEET showed activity similar to solvent control. These data indicated that $C_{12:0}$ is not only as active as DEET at 25 nmol/cm^2 but has higher activity than DEET at lower concentrations.

Based on Fig. 1, it appeared that there may be a relationship between fatty acid chain length and biting deterrence. Therefore, we decided to examine the relationship between fatty acid chain length and calculated Log P (Cantrell et al. 2001) and mosquito biting deterrent activity. Log P is a ratio of the concentrations of a compound in two solutions, octanol and water. Log P determinations were calculated using Advanced Chemistry Development (ACD/Labs) Software Version 11.02. A plot of Log P for both saturated and unsaturated fatty acids versus BDI showed a peak in biting deterrent activity near Log $P = 5$ (Fig. 5). The equation for the overlaid second order polynomial is $y = -0.0588x^2 + 0.5543x - 0.4228$.

The MED for repellency of four fatty acids and the ratio of MED to that of DEET are reported for three species of mosquitoes in Table 1. The mid chain length fatty acids $C_{11:0}$, $C_{11:1}$, $C_{12:0}$, and $C_{12:1}$ exhibited repellency toward *Ae. aegypti*, but none had MED values as low as DEET. In general, a lower surface concentration of compound was needed to repel *An. quadri-*

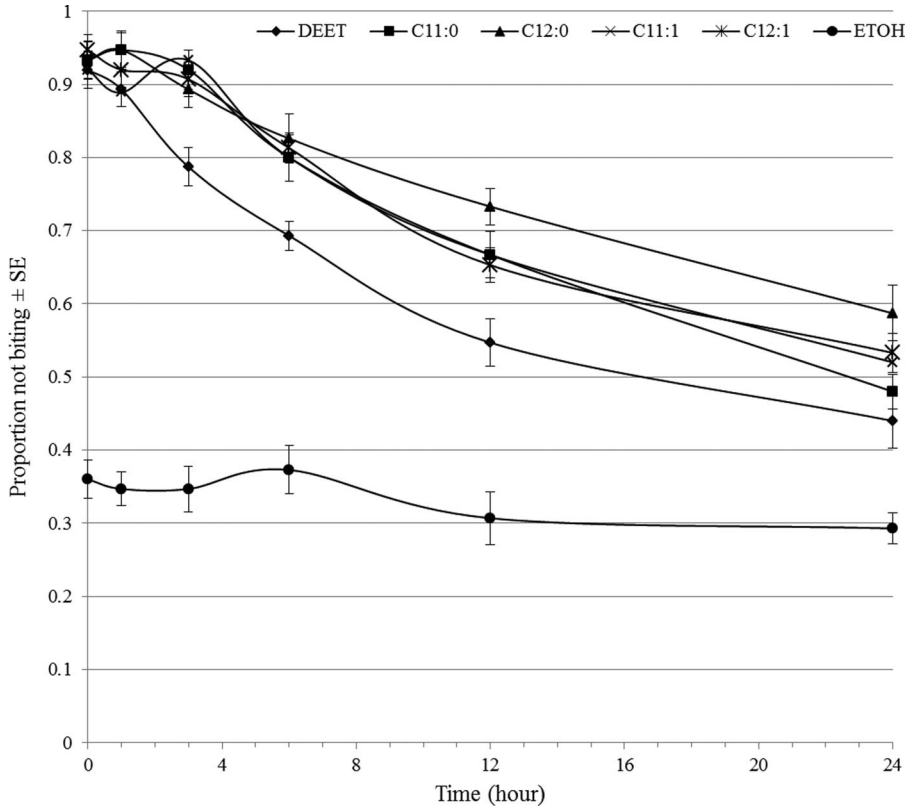

Fig. 3. Duration of biting deterrent effects for *Aedes aegypti* to $C_{11:0}$, $C_{12:0}$, $C_{11:1}$, and $C_{12:1}$ at 25 nmol/cm^2 using DEET at 25 nmol/cm^2 as positive control and ethanol as solvent control. Females were tested at 0, 1, 3, 6, 12, and 24-h posttreatment.

maculatus than was required to repel the other two species, with the exception of DEET where the MEDs for repellency were not significantly different from those calculated for *Ae. aegypti* and *An. quadrimaculatus.* The standard repellent DEET was required in much higher surface concentration to repel *An. albi-*

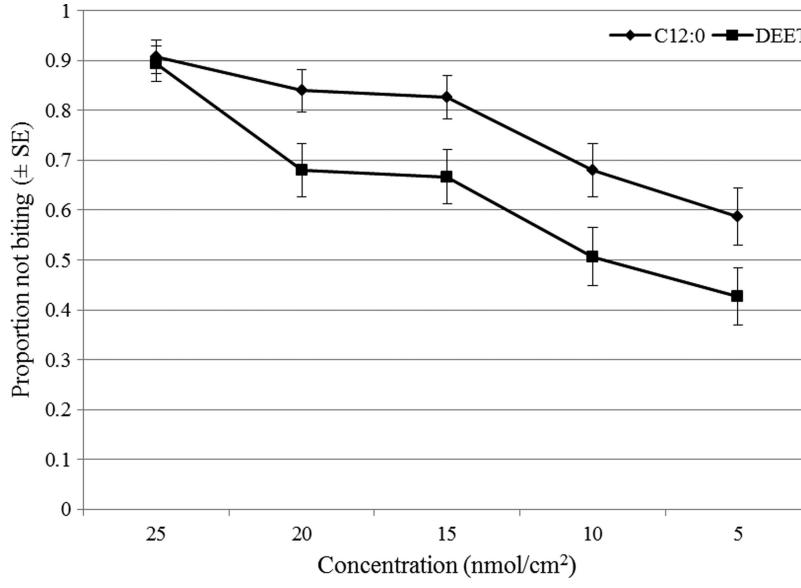

Fig. 4. Dose–response of biting deterrent effects for *Aedes aegypti* to $C_{12:0}$ and DEET using ethanol as solvent control.

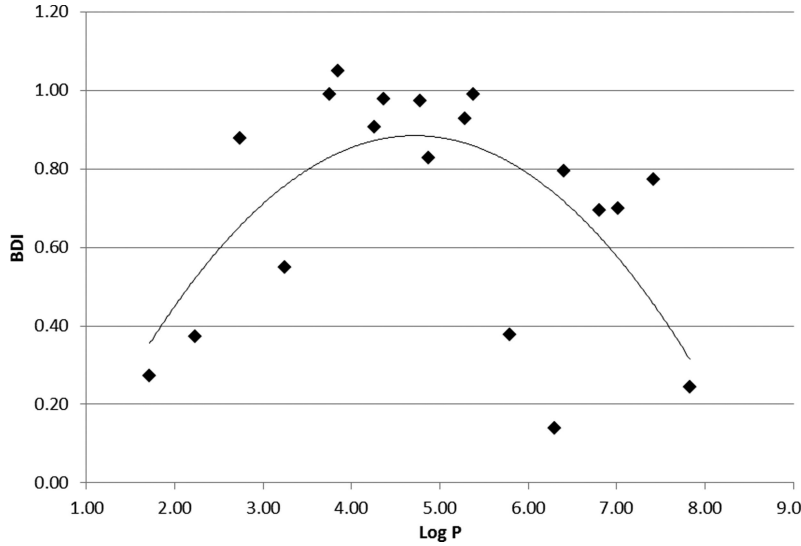

Fig. 5. BDI values for saturated and unsaturated fatty acids against calculated Log P (unit less) with overlaid second order polynomial trendline ($y = -0.0588x^2 + 0.5543x - 0.4228$) for emphasis.

manus, a result previously documented by Klun et al. (2004). The most active compound in the "cloth patch assay" was $C_{11:1}$, with a MED value of 445 ± 130 nmol/cm^2 for *An. albimanus*, a concentration 1.4 times higher than DEET. The ratios for the MEDs of fatty acid:DEET were larger for each fatty acid against *Ae. aegypti* compared with each acid against the Anophelines.

Discussion

Dodecanoic acid and undecanoic acid have long been known to be active against *Ae. aegypti* and *An. quadrimaculatus* on both cloth and skin. In an original study (United States Department of Agriculture [USDA] 1954); dodecanoic acid was significantly less repellent against *Ae. aegypti* and *An. quadrimaculatus*, and 10-undecenoic acid tested as the sodium salt was ineffective against *Ae. aegypti.* Hwang et al. (1982) reported that longer chain fatty acids were at least as active or more active than shorter chain acids in repelling ovipositing females in different mosquito species; in particular $C_{8:0}$ to $C_{10:0}$ were more active than $C_{2:0}$ to $C_{6:0}$.

Analysis of the relationship between lipophilicity (Log P) and mosquito biting deterrent activity may serve as a useful parameter in the evaluation of potential mosquito biting deterrent compounds. Our data suggest that a Log *P* value between 4 and 5 may be a useful indicator or predictor of mosquito biting deterrent activity for saturated and unsaturated fatty acids. This is somewhat different from other compounds, for example, pesticides and many pharmaceuticals, where potentially useful compounds rarely exceed a Log P of five (Lipinski et al. 1997, Tice 2001).

While compounds are still screened for residual activity as has been done historically, a greater emphasis is now placed on rapid determination of the potency of potential repellents. Improvements in knowledge of formulation chemistry have made possible the use of more volatile repellents that evaporate more slowly via formulation (N′guessan et al. 2007). For this reason, candidate repellents are now initially screened for efficacy at a "high" concentration, and if these pass, they are secondarily screened for an estimation of the MED, which is the concentration at which 100% repellency fails and bites are received by the test subjects (Katriztky et al. 2010). This is the first

Table 1. Minimum effective dosage of DEET and select saturated and unsaturated fatty acids required to repel mosquitoes using an in vivo "cloth patch" assay

Treatment	Species					
	Ae. aegypti	R	*An. albimanus*	R	*An. quadrimaculatus*	R
Undecanoic acid	124 ± 1	2.6	471 ± 139	1.5	120 ± 56	2.2
Dodecanoic acid	649 ± 193	14.8	1246 ± 221	4.1	145 ± 51	2.9
10-undecenoic acid	297 ± 43	6.2	445 ± 130	1.4	116 ± 40	2.1
11-dodecenoic acid	342 ± 42	7.7	1513 ± 569	5.0	141 ± 36	2.7
DEET	46 ± 5	1.0	316 ± 82	1.0	54 ± 11	1.0

Mean MED ± SE (nmol/cm^2). R, Ratio of treatment MED: DEET MED; $N = 9$ for *Ae. aegypti*, $N = 6$ for *An. albimanus*, and $N = 7$ for *An. quadrimaculatus*.

peer-reviewed study on the "biting deterrent" effects of fatty acids with a wide range of chain lengths against mosquitoes. These data showed promise as biting deterrents that supported exploration of formulations of the mid chain length fatty acids ($C_{10:0}$ to $C_{13:0}$), both saturated and unsaturated, that can be effectively used in integrated vector management programs. It is not known if a combination of these fatty acids might provide a more potent blend at this time. Further studies are needed to evaluate the efficacy of blends of some of these fatty acids.

In assays with human subjects, $C_{11:0}$ was more potent than $C_{12:0}$ fatty acids while in in vitro assays both showed similar levels of activity. It is important to note that the concentrations of compounds used in each type of assay were also different because these two assays measure different types of behavior. These differences contribute to the higher concentrations of compounds needed to repel mosquitoes in a "cloth patch" assay. In a module based system mosquitoes are contained in enclosed spaces that minimize the host-seeking aspect and maximize the host-feeding aspect; however, unless conducted in vivo, the attractance is imperfect because a feeding solution is likely to be less attractive than skin odors. The cage used in the cloth patch assay is larger and does require some host-finding ability by the mosquitoes to locate the source of escaping host odors, and then to land and feed through the cloth. Understanding the parameters that affect repellency in in vitro screening can minimize the false positive responses, while rigorous standards in later experimentation facilitate identification of potential new repellents for field evaluation. All bioassays have their limitations but the bioassay procedures based on biological relevance can facilitate the comparison of the results from different bioassays (Barnard et al. 2007). It should also be kept in mind that use of cloth is still not an exact predictor of compound efficacy on the skin, so the results obtained here should be verified eventually through the use of a true topical repellent assay.

There is a clear difference between results of biting deterrence from the module system and the results obtained from the cloth patch assay. These are two different bioassay systems and cannot be directly compared. Some of these differences can be attributed to the closed space of the K & D module system and the potential for increased concentration of the volatiles within the module. This may produce overestimation of deterrent efficacy that is likely to become increasingly problematic as the volatility of the test compound is increased. Although the levels of biting deterrence in the K & D system did not directly translate quantitatively into repellency in the cloth patch bioassay, >95% of the compounds which showed biting deterrence in the K & D bioassay have also shown repellency in cloth patch bioassays. Perhaps "in other words, what was active in one test system, also affected mosquito behavior in the other." This is a critical point as it suggests that the screening of compounds with a high throughput system like the K & D module is both sensitive and specific, at least with respect to the ability (or not) of a chemical to affect behavior. Whether or not this observation has validity outside of our results, for example, for different chemical classes, species of biting insect, or behavioral endpoints of interest, remains to be seen.

We suggest that future work should systematically evaluate these acids, including more "real world" scenarios, with the aim of developing novel products and/or strategies targeting mosquitoes and other biting arthropods. This is an important conclusion that can make a basis for finding better use of these acids in future endeavors to develop management strategies against mosquitoes.

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